

Evolutionary dynamics of imatinib-treated leukemic cells by stochastic approach

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Abstract

The evolutionary dynamics of a system of cancerous cells in a model of chronic myeloid leukemia (CML) is investigated by a statistical approach. Cancer progression is explored by applying a Monte Carlo method to simulate the stochastic behavior of cell reproduction and death in a population of blood cells which can experience genetic mutations. In CML front line therapy is represented by the tyrosine kinase inhibitor imatinib which strongly affects the reproduction of leukemic cells only. In this work, we analyze the effects of a targeted therapy on the evolutionary dynamics of normal, first-mutant and cancerous cell populations. Several scenarios of the evolutionary dynamics of imatinib-treated leukemic cells are described as a consequence of the efficacy of the different modeled therapies. We show how the patient response to the therapy changes when an high value of the mutation rate from healthy to cancerous cells is present. Our results are in agreement with clinical observations. Unfortunately, development of resistance to imatinib is observed in a proportion of patients, whose blood cells are characterized by an increasing number of genetic alterations. We find that the occurrence of resistance to the therapy can be related to a progressive increase of deleterious mutations.

PACS numbers: 87.10Mn, 87.10.Rt, 87.23Kg, 87.19.xj

Keywords: Stochastic dynamics, Cancer evolution, Complex systems

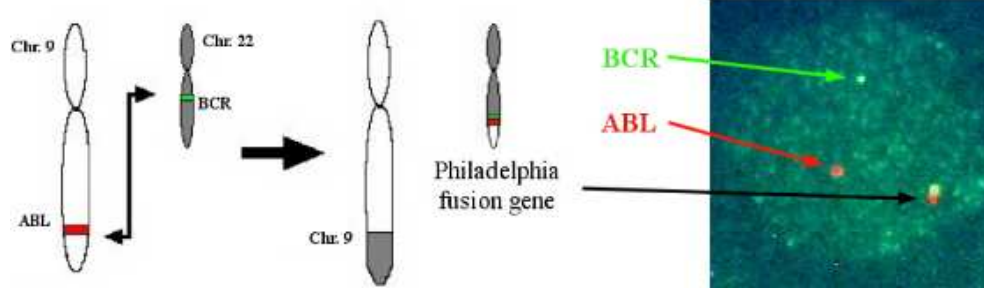


FIG. 1: Schematic representation of the Philadelphia translocation (left) and detection mechanism by Fluorescence Interphase in Situ Hybridization (FISH) (right).

I. INTRODUCTION

In the last decade cancer dynamics and tumor growth models have been attracted an increasing interest [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. Among many different kind of human cancer, a recent investigation of the dynamics of Chronic Myeloid Leukemia (CML) has provided the first quantitative insights into the *in vivo* kinetics [4]. In the bone marrow of patients affected by CML too many myeloid cells (one of the main types of white blood cells) are produced and released into the blood when they are immature and unable to work properly. These immature cells (called blasts) cannot do the work of normal white blood cells, and this leads to an increased risk of infections. Furthermore, they fill up the bone marrow making difficult the production of enough healthy red cells and platelets (see Ref. [20] for a recent review). CML cells are characterized by a specific chromosomal abnormality: the Philadelphia (Ph) chromosome [21, 22, 23], which is created by a reciprocal translocation between part of the BCR ("breakpoint cluster region") gene from chromosome 22 and part of the ABL gene on chromosome 9 (ABL stands for "Abelson", the name of a leukemia virus which carries a similar protein) (Fig. 1). BCR-ABL oncogene activates a number of cell cycle-controlling proteins (p210 or sometimes p185) and enzymes (tyrosine kinase), speeding up cell division. Moreover, it inhibits DNA repairing, causing genomic instability. Until recently, the only successful treatment of CML was to destroy the patient's bone marrow and then restore blood-cell production by infusing stem cells from the bone marrow of a healthy donor [24, 25, 26]. In the late 1990s, Novartis Pharmaceutical discovered a very efficient tyrosine kinase inhibitor: STI-571 (imatinib, Gleevec/Glivec). Subsequent clinical trials demonstrated that Gleevec inhibits the proliferation of BCR-ABL-expressing

hematopoietic cells, turning off the signal produced by the Ph chromosome [27, 28, 29, 30]. CML represents the first human cancer in which molecularly targeted therapy leads to a striking clinical response. Although it does not eradicate CML cells, it greatly limits the growth of the tumor clone. Unfortunately, acquired resistance to Gleevec develops in a substantial fraction of patients [31]. The basis for resistance is a genetic change in the BCR-ABL gene itself: in particular point mutations within the protein tyrosine kinase domain [32, 33].

The evolutionary dynamics of cancer beginning and progression has been theoretically approached in several works with mathematical deterministic equations [4, 5, 12] or with stochastic models [1, 3, 6, 7, 9, 11, 13, 14, 16, 19]. These works describe the temporal evolution of the level of BCR-ABL positive cells, experimentally observed in patients treated with Gleevec, in terms of a partial or total failure of the drug efficacy on cancer stem cells. This would explain the difficulty to completely eradicate the cancer. Instead of a general insensitivity of stem cells to imatinib, a selective effect on proliferative cells, as a process of competition between healthy and cancerous stem cells for appropriate niches, is also proposed [15, 19]. On the other hand, Gleevec effect on leukemic progenitors [34] appears to be strictly related to the rapid enhancement of the number of blasts after a therapy stop.

In this work we investigate the evolutionary dynamics of leukemic cells by simulating the stochastic behavior of cell division and mutation in a system of initially normal blood cells which can be affected by a double genetic mutation and transformed in cancerous cells. In order to simulate the random process of cell selection for reproduction, mutation and death (each process being guided by an appropriate fitness or event rate) we adopt a Monte Carlo approach, as already done by several authors in theoretical cancer studies (see refs. [3, 8, 12, 13, 14, 19, 35, 36, 37], to cite a few). The aim of this work is to explain the several observed scenarios of CML patient response to imatinib by modeling a therapy side effect of enhancement in the number of deleterious mutations from healthy to leukemic cells. In this framework, we also investigate the development of resistance to the therapy and the subsequent relapse. In sect. II we describe the model and give the details of the simulation process; results are reported in sect. III and conclusions are drawn in sect. IV.



FIG. 2: Logical map of the evolution of the blood cells from normal type (green box) to first-mutants (yellow box) and finally to leukemic cells (red box).

II. STOCHASTIC MODEL

The Ph-translocation is a particular form of chromosomal instability (CIN) [38, 39], that refers to increased rates of gaining or losing whole chromosomes or arms of chromosomes. Several hundred genes contribute to maintaining the stability of chromosomes during cell division; mutations in such genes can trigger the CIN phenotype. The basic idea that cancer arises when a single cell experiences multiple mutations has been confirmed by numerous studies on cancer genetics [40, 41, 42].

In this work we apply a Monte Carlo approach to study the dynamics of a finite population of N replicating cells under the effect of two sequential mutations. The total number of cells is kept constant during the time evolution, as it can be reasonably assumed for a blood cancer. In our simulations, we have chosen $N = 10^4$ cells. This value is several orders of magnitude lower than the typical total contents of blood cells in humans, but it is great enough for the statistical study of the cancer development in a single blood compartment. Our model contains three types of cells, denoted by 0, 1, and 2. Cell population of type 0 can mutate to cells of type 1 at a rate M_{01} and type 1 cells mutate to type 2 at a rate M_{12} . The reproductive rates (fitness) of cell types 0, 1 and 2 are labeled by F_0 , F_1 , F_2 , respectively. In Fig. 3 is shown a logical map of the evolution of the blood cells from normal to cancerous types. We do not consider back mutations and neglect direct transitions from healthy (type 0) to leukemic cells (type 2).

The stochastic dynamics of the cancer evolution is modeled by assuming that cells reproduce asynchronously. This means that each elementary step of the stochastic process consists of a birth and a death event (Moran process [43]). For the reproduction process, one of the N cells is randomly chosen proportionally to the fitness. It will give rise to an offspring (a new cell of the same type) subject to possible mutation. The exact sequence of logical steps for a single reproduction event is described by the flowchart shown in Fig. 3.

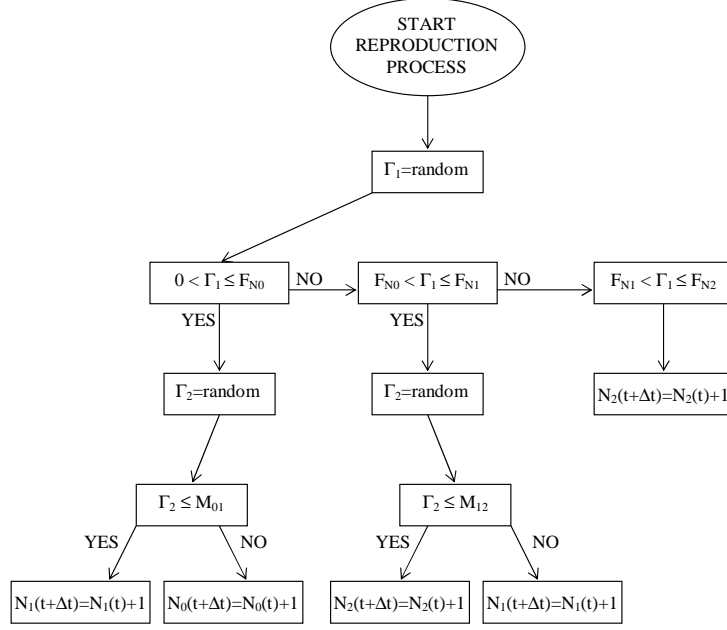


FIG. 3: Flowchart of the reproduction event in a single time step. F_{N_i} (with $i = 0, 1, 2$) are the normalized fitness rates; M_{01} , M_{12} are mutation rates for transitions from type 0 to type 1 cells and from type 1 to type 2 cells, respectively.

Within the same time step a death event occurs, being one of the N cells chosen at random to be eliminated (see Fig. 4). This guaranties that the total population size remains constant. At time $t=0$, all N cells are of 0-type. After some time, a single mutant cell of type 1 is generated. This cell leads to a lineage of type 1 cells that could mutate to type 2 or go to extinction before a second mutation event from type 1 to type 2 takes place. In order to give a statistical significance to our descriptions, every simulation is repeated 500 times and all the results reported in the next section are based on ensemble averages. Time is measured in units of cell divisions. A time scaling, described in the next section, is applied in order to show the results in day time units.

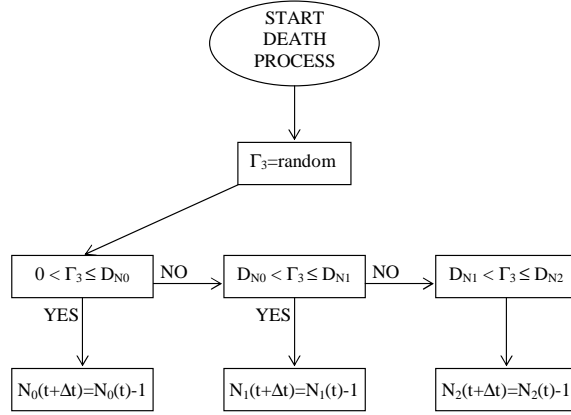


FIG. 4: Flowchart of the death event in a single time step. D_{N_i} (with $i = 0, 1, 2$) are the normalized death rates.

III. RESULTS

A. Therapy effects on cancer evolutionary dynamics

The first case under investigation is the stochastic evolution of the three types of cell populations in the absence of any therapy. Real values of mutation rates from normal cell to leukemic type are not available because nobody knows with certainty how long is the latency of the CML. We have chosen the mutation rates M_{01} and M_{12} equal to 0.001 and 0.02, respectively. These values are one order of magnitude greater than the mutation rates adopted elsewhere [3] because, in this study, we are not interested in the average waiting time before the disease make its first appearance, but in the subsequent dynamic evolution. Normal cells (green line in Fig. 5a) experience the first mutation after some time interval. If the intermediate-type cells (yellow line) survive for a sufficient long time, a second-type mutation can cause the birth of cancerous cells (red line). In our code the fitness F_0 and F_1 are set equal to 1, while the fitness of type 2 cells has been reasonably assumed 10 times that of the other two populations. For this reason, the number of cancerous cells rapidly increases to the total initial value N of normal cells.

The effect of an ideal therapy is modeled by reducing the reproductive capability of the only type 2 mutated cells. We know that an imatinib-based therapy can also induce the CML cells apoptosis [44, 45], but, at this stage, we do not include this effect in our

model. Therefore the death rates D_0 , D_1 and D_2 , associated to type 0, type 1 and type 2 cells, respectively, are left equal to each other. Since a successful therapy requires a basic reproductive ratio of cancerous cells lower than 1 [12], we model the therapy effect by lowering the fitness parameters F_2 to 0.7. All our simulations start with the patient developing CML in the absence of therapy; when the number of leukemic cells exceeds the threshold value $N/3$, the therapy is activated. In fig. 5b we can see that, after an initial increase of the number of leukemic cells, the effect of an ideal therapy is to completely eradicate the number of mutated cells and favor the restoring of normal cells. The time scaling from cell division to days is performed by assuming a complete restore of healthy cells in almost 100 days, as experimentally observed in clinical cases of optimal therapy response [4, 15].

For the study of real cases of therapy effects on cancer evolution, we have supposed that the cell system could react to the drug administration by activating multiple genetic changes that cause an enhancement of the mutation rates from normal to cancerous cells. This assumption is supported by the experimental evidence that certain mutations increase the rate at which subsequent mutations occur [46, 47]. This secondary effect of the therapy is investigated by modeling an increase of M_{01} and M_{12} at four different levels, as summarized in table I. In the case 1 of real therapy (Fig. 5c) we have increased both mutation rates by a factor 10. While the extinction effect on type 2 cells is almost unchanged with respect to the previous case (Fig. 5b), the first-mutant cells disappear very quickly because of the increased value of M_{12} . When the mutation rate M_{12} is doubled and M_{01} progressively increased (cases 2, 3 and 4 in table I), an effect of retard is observed in the recovery of healthy cells (Figs. 5d and 5e) and, in the worst case, a failure of the therapy itself is present (Fig. 5f). This means that, even if the therapy works properly by inhibiting the reproduction of leukemic cells, the cancer proliferation can still occur because of an increase of disadvantageous genetic mutations.

B. Development of resistance to the therapy

Several cases of acquired resistance to the therapy has been observed in CML patients. In recent experimental works genetic mutations are observed in a significant fraction of resistant patients [48]. An increase of the mutation rates could represent the natural evolution of

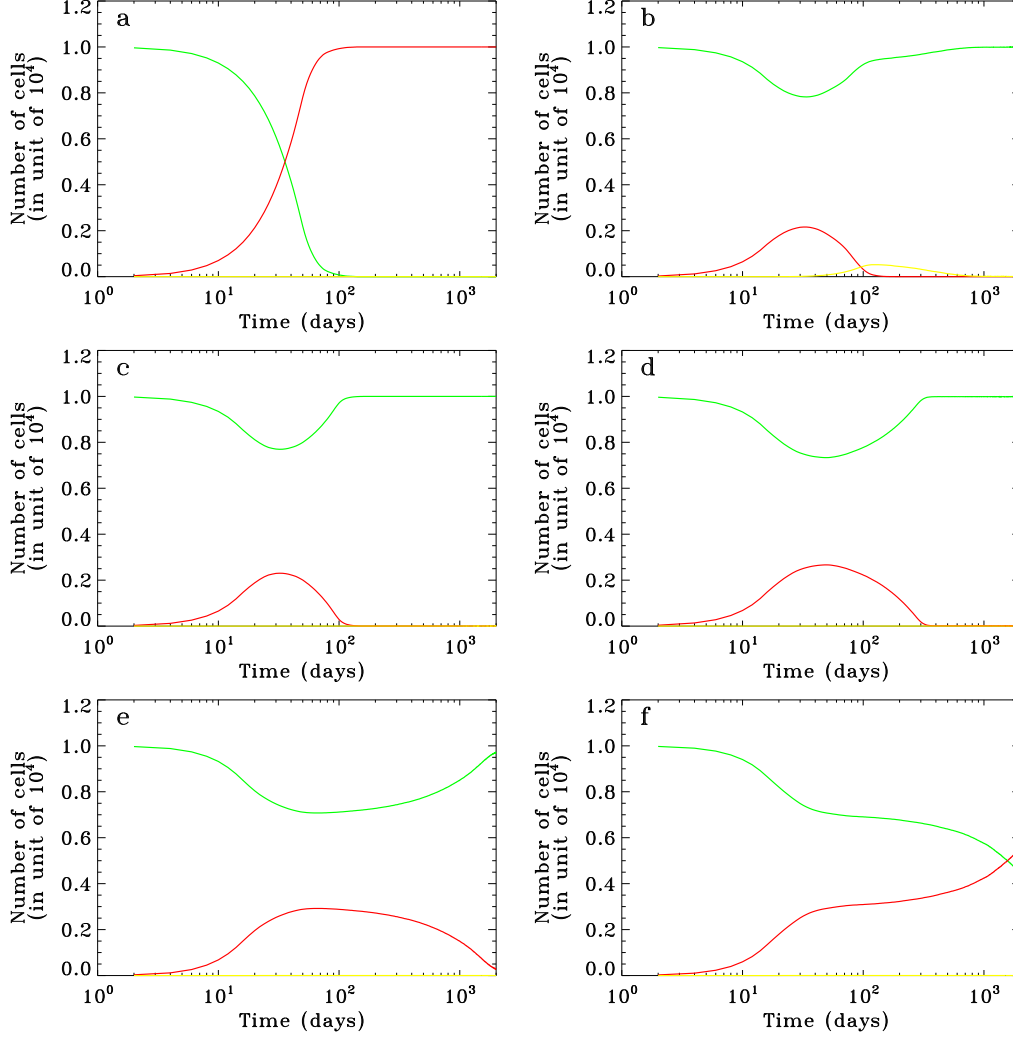


FIG. 5: Evolutionary dynamics of cancerous cells. The green line indicates the behavior of healthy cells, yellow line that of first mutation cells and red line that of second mutation cancerous cells: (a) without any therapy; (b) including the effects of an ideal therapy with an activation threshold of $N/3$ cancerous cells; (c, d, e, f) a real therapy with increasing mutation rates (see table I).

TABLE I: Table of model parameters.

	No therapy	Ideal therapy	Real therapy (Case 1)	Real therapy (Case 2)	Real therapy (Case 3)	Real therapy (Case 4)
F_2 (cancerous cell)	10.0	0.7	0.7	0.7	0.7	0.7
M_{01}	0.001	0.001	0.01	0.10	0.13	0.14
M_{12}	0.02	0.02	0.20	0.40	0.40	0.40

cancerous cells as a defense from the action of the therapy itself. In the previous section we have shown that a therapy based on the drug inhibitory action of leukemic cell reproduction mechanism can fail, if the therapy also causes an increase of the mutation rates from normal cells. We investigate the development of resistance to the therapy by modeling a time-dependent increase of both mutation rates. We have reasonably decided to apply a time delay of 100 days, from the beginning of the therapy, before M_{01} and M_{12} start to grow up. This value has been chosen on the basis of experimental findings regarding an initial response of the patient to the therapy before the first appearance of the resistant clone. We have modeled a linear increase of M_{01} and M_{12} with slopes of 0.001/day and 0.002/day, respectively, for the subsequent 240 days, until M_{01} reaches the value 0.22 and M_{12} 0.50. These values are assumed as upper limits for M_{01} and M_{12} , because greater mutation rates do not significantly change the already dramatic cutoff of healthy cells and the rapid increase of the cancerous cells. The time dependent behavior of M_{01} and M_{12} are plotted in Fig. 6a. The evolutionary dynamics of the three cell populations in CML patients, treated with a therapy causing a progressive increase of mutation rates, are shown in Fig. 6b.

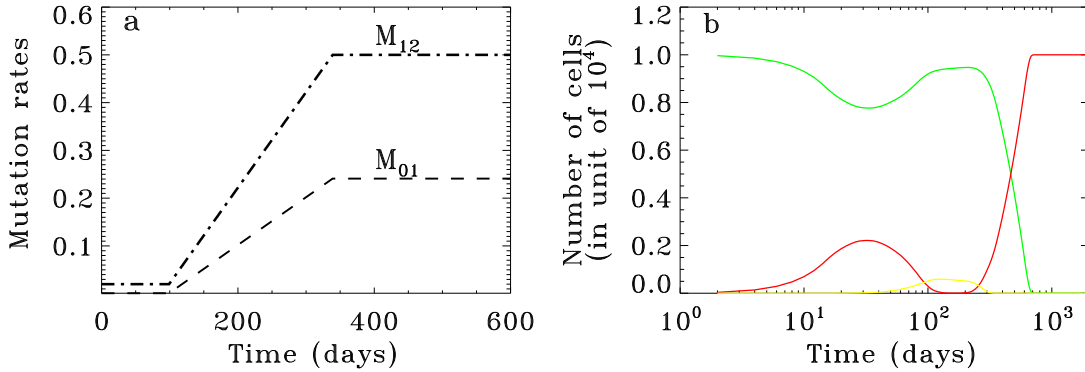


FIG. 6: (a) Changes on mutation rates with the time. (b) Evolutionary dynamics of cancerous cells: development of resistance. The green line indicates the behavior of healthy cells, yellow line that of first mutation cells and red line that of second mutation cancerous cells

After the therapy activation, the cancerous cells initially respond to the medicine, starting a decreasing trend towards a total disappearance. When the mutation rates start to grow, the number of cancerous cells starts to increase again, exceeding the previous therapy activation level and quickly reaching the total value N . Fig. 6b represents a clear example of acquired resistance that brings the system to a totally cancerous cell condition in spite of the presence

of therapy.

IV. CONCLUSIONS

Chronic myeloid leukemia is a blood cancer that causes an overproduction of immature white cells. In this paper we provide a statistical description of the effects of an imatinib-like based therapy on the evolutionary dynamics of normal, first-mutant and cancerous cell populations in a stochastic model of chronic myeloid leukemia. Imatinib strongly limits the reproduction of only cancerous cells, permitting a cancer targeted therapy. Unfortunately, acquired drug resistance is the major limitation for the successful treatment of cancer. In fact, several patients, developing resistance to the therapy, show a relapse towards the accelerated phase and blast crisis which are strongly characterized by genetic mutations.

In this work, we study the effect of different mutation rates on the somatic evolution of cancer. We find that the response to the therapy, in terms of a permanent reduction of malignant cells, depends on both the inhibitory capability of the medicine and on the levels of mutation rates from normal cells to leukemic types. In the best cases, a total (but temporary) recovery is usually obtained in almost 100 days, as experimentally observed in clinical cases. Nevertheless, we show that an enhancement of the mutation rates from healthy cells to type 1 (first-mutants) and from type 1 to cancerous cells, caused by genetic alterations, can retard the complete depletion of leukemic cells. Our modeled evolutionary dynamics of leukemic cells are in agreement with the different temporal response of patients treated with imatinib observed in clinical studies, as summarized in Fig. 1b of Ref. [15].

Moreover, we point out that, in the worst cases, this enhancement of mutation rates can bring the cell system to an acquired resistance to the therapy. In fact, after an initial response, we find a dramatic enhancement of leukemic cell abundance even in the presence of a significant reduction of cancerous cell fitness induced by the therapy. Our theoretical results are further supported by the experimental evidence of an increase of genetic mutations in patients which show acquired resistance [48].

Our work provides a step towards the construction of a comprehensive theory for the dynamics of cancer evolution. A more realistic description of therapeutic scenarios, however, needs to take into account (a) the therapy effect on the leukemic cell death rate, (b) tunneling phenomena from type 0 to type 2 cells and (c) a dose dependent response of patients to the

therapy. All these topics will be investigated in a next work.

Acknowledgments

This work was supported by MIUR and CNISM-INFM.

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